Remarks

This amendment is filed in response to the final Office Action mailed on April 27, 2005. This response is filed with a Request for Continued Examination.

Claims 37, 43 and 55 have been amended. Support for the amendments to claims 37 and 43 is found in the last complete paragraph on page 9 of the specification. Claim 55 was amended to be consistent with the claims from which it depends. No new matter has been added.

Objection Under 37 CFR 1.75(c)

Claim 43 was objected to under 37 CFR 1.75(c) as being in improper dependent form for failing to further limit the subject matter of the previous claim. The Examiner argues that the requirement in claim 43 for the antigen and the non-toxic double mutant form of pertussis toxin to be "administered at the same time" does not limit the requirement in claim 37 that the two are "co-administered".

Applicant has amended claims 37 and 43 as shown above to address the objection. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the objection under 37 CFR 1.75(c).

Rejections Under 35 U.S.C. 103(a)

A. The Examiner rejected claims 37, 39, 41-44, 46 and 55 under 35 U.S.C. § 103 as unpatentable over Nencioni et al. or Podda et al. in view of Capiau et al., Tamura et al. and Honda et al. Claim 45 is rejected further in view of Halpern et al. Reconsideration of the rejection is respectfully requested in view of the following evidence that the claimed invention is unexpected and therefore nonobvious.

The claimed invention is concerned with a method of using a non-toxic double mutant of pertussis toxin as a mucosal adjuvant, i.e., as a substance that stimulates or enhances a protective immune response to an antigen that is administered to a mucosal surface with the mutant pertussis toxin. The pertussis toxin has mutations at positions 9 and 129 of the S1 subunit that render it non-toxic by inactivating the ADP-ribosylating enzymatic activity of the native toxin.

The Nencioni and Podda references teach that a double mutant of pertussis toxin is an effective antigen. The Nencioni and Podda references neither teach nor suggest that this mutant toxin is an effective adjuvant, more particularly an effective mucosal adjuvant. Moreover, the lack of adjuvant activity is reinforced by the inclusion in the composition of the trial vaccine of a well-known adjuvant. As described in the Podda reference on page 862, the vaccine preparation as including 0.5 mg of aluminum hydroxide (alum). Based on the lack of description of the double mutant of pertussis toxin as an adjuvant and on the inclusion of a well known adjuvant in the composition used in these references, one of ordinary skill in the art, in reading these references, would not conclude that the double mutant of pertussis toxin has <u>any</u> adjuvant activity.

Furthermore, Podda indicates that the method of vaccination was intramuscular injection (see p. 862), not mucosal administration. Thus, there is no teaching or suggestion in the Nencioni and Podda references of a method for stimulating or enhancing a protective immune response to an antigen using a double mutant pertussis toxin as an adjuvant by mucosal administration.

The other references cited by the Examiner do not provide the elements missing from the teachings of the Nencioni and Podda references, as it described in greater detail below. Thus the combination of references does not render the claimed invention unpatentable as obvious.

The Capiau et al. reference teaches that a pertussis toxin S1 subunit having a modification of the tryptophan residue at position 26. Capiau teaches that this modified protein has greatly reduced toxicity while retaining its ability as an antigen to elicit a protective immune

response. See page 4, lines 39-41 of Capiau. While the modified protein is enzymatically inactive, it nevertheless can be recognized by anti-pertussis toxin antibodies. See page 4, lines 59-61.

Capiau does not teach or suggest that the modified protein has adjuvant activity, however. This is also true for modifications having further mutations, including those at the positions recited by Applicant in the claims.

At the time that the instant application was filed, a skilled person thought that the adjuvant activity of pertussis toxin was likely to be inseparable from its enzymatic activity, and it was therefore expected that inactivating the enzymatic activity of the toxin would also inactivate its adjuvant activity.

Evidence that the adjuvant activity of pertussis toxin was perceived to be likely to be inseparable from its enzymatic activity is provided by Roberts et al. (1995) Infection and Immunity 63, 2100-2108 (which is already of record). See the right column on page 2106 of Roberts et al., where it is stated that:

> "The mechanism(s) by which PTX exerts adjuvanticity is unknown but is thought to require an enzymatically active S1 subunit" (emphasis added.)

Roberts et al. goes on to describe one of the experiments which led to the belief that adjuvanticity was thought to require an enzymatically active S1 subunit. In particular, Roberts et al. describes an experiment in which it was shown that heat-killed whole cells of *B. pertussis* strains which had their S1 subunit gene deleted or which had an insertion in S1 resulting in a 90% drop in ADP-ribosylating activity did not enhance the serum antibody response to ovalbumin, whereas killed cells prepared from strains with wild-type PTX genes did (Roberts et al., page 2106, right column).

However, Applicant has found the opposite to be true. Applicant found that, if anything, the mutant enzymatically inactive pertussis toxin as recited in the claims is a more effective

mucosal adjuvant than the wild-type toxin. That was an unexpected result, which is indicative of nonobviousness.

Further evidence that the adjuvant activity of pertussis toxin was likely to be inseparable from its enzymatic activity is provided by Holmgren et al. (1993) Vaccine 11, 1179-1184 (previously supplied). Holmgren et al. is a review of the use of cholera toxin and the B subunit of cholera toxin as an oral-mucosal adjuvant and antigen vector system. Holmgren et al also discusses the heat-labile toxin (LT) from *E. coli*. Pertussis toxin in closely related to both cholera toxin and heat-labile toxin in the sense that all three are bacterial toxins, all three have an AB_5 subunit structure (A = active subunit, B = binding subunit), the A subunits of all three have ADP-ribosylating enzymatic activity and all three have adjuvant activity. Thus, persons skilled in the art believed that what was true of one of the three toxins was generally also likely to be true of the other two.

Holmgren et al. contains a section entitled "Can adjuvanticity be separated from enterotoxicity?" (see pages 1182-1183) The experiments described in this section of Holmgren et al. clearly suggest that the answer to this question is "no". The results presented in Holmgren show that CT (cholera toxin) and LT (heat-labile toxin) are effective adjuvants but that recombinant B subunit of CT ("rCTB") is not an effective adjuvant. The conclusion that Holmgren et al. reached was that:

> "This adjuvant activity appears to be closely linked to the ADP-ribosylating action of CT and LT associated with enhanced cyclic AMP formation in the affected cells, and that it may prove difficult to eliminate the enterotoxic activity without loss of adjuvanticity." (See the abstract of Holmgren et al.)

The Examiner argues that the art had demonstrated that adjuvant activity was independent of enzymatic activity and cites Honda et al. in support of this argument. However, for reasons which are explained below, the work of Honda et al. and other similar work was discredited shortly after the publication of Honda et al.

Honda et al. allegedly shows that the B subunit of pertussis toxin is a mucosal adjuvant in the absence of the S1/A subunit. However, it was subsequently shown in the art that this is not correct. In particular, it was subsequently shown that allegedly pure preparations of the B subunit such as that described in Honda et al. were in fact contaminated by very small amounts of active pertussis toxin containing the S1/A subunit and that it was these very small amounts of active pertussis toxin that were responsible for the adjuvant activity. When studies similar to that reported in Honda et al. were repeated using very pure B subunit (e.g., B subunit produced from cells not expressing any active subunit), it was found that there was no adjuvant activity. See, for example, Holmgren et al., page 1182, left column, last complete paragraph, where it is stated that:

> "Commercial preparations of CTB as used in the 'positive' studies regularly contain 0.1-2% of contaminating CT holotoxin, while we have used a highly purified CTB with no detectable CT (<0.0001%) or recombinant CTB from a genetically CT-deleted *V. cholerae* strain producing plasmid encoding CTB. On the other hand, when we added 0.1% CT to CTB (0.1 μg to 10 μg as the oral immunising dose) a strong adjuvant effect was observed."

Similarly, Tamura et al. (1994) Vaccine 12, 419-426 (previously provided) reported that addition of a trace amount of CT to CTB (or LTB) converted a preparation devoid of adjuvant activity into one exhibiting potent adjuvant activity. See the last sentence of the abstract of Tamura et al., which states that:

> "These results suggest that CTB (or LTB) containing a trace amount of CT (about 0.1%) can be used practically as a potent adjuvant for nasal vaccination of humans against influenza."

In assessing the art, it is important not to confuse adjuvant activity with antigen activity. As the Examiner will appreciate, adjuvant activity refers to the ability of a substance to stimulate or enhance the immune response against a co-administered substance. In contrast, an antigen merely induces an immune response against itself.

Finally, Applicant emphasizes that it is difficult to predict whether a substance will function as an adjuvant, given that the precise manner in which adjuvants work is something of a

mystery. It could and would not have been predicted that the non-toxic double mutant of pertussis toxin recited in the claims would be a highly effective adjuvant and would, if anything, be a more effective adjuvant than the native form of the toxin. There is nothing in the combination of references cited by the Examiner that clarifies this aspect. Thus, the skilled person would not have had a reasonable expectation of success given the knowledge in the art and the teachings of the cited combination of references.

In conclusion, the combination of the prior art cited by the Examiner does not provide the elements of Applicant's claimed invention. In particular, Nencioni et al., Podda et al. and Capiau et al. teach antigen activity of the mutant pertussis toxin but not any adjuvant activity (particularly mucosal activity), and the results of Honda et al. have been discredited with respect to their teachings of adjuvant activity attributable to pertussis toxin B subunit. The teachings of the other cited references do not, in combination, provide the elements missing from Nencioni et al., Podda et al., Capiau et al. and Honda et al., and furthermore do not provide a reasonable expectation of success for one skilled in the art for using a pertussis toxin double mutant as a mucosal adjuvant.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. 103(a).

B. The Examiner rejected claim 45 under 35 U.S.C. § 103 as unpatentable over Nencioni et al. or Podda et al. in view of Capiau et al., Tamura et al. and Honda et al., and further in view of Halpern et al. Applicant respectfully requests reconsideration of the rejection.

Claim 45 is dependent upon claim 37. Applicant submits that claim 45 is patentable for the same reasons as given above for claim 37. The disclosure by Halpern of tetanus toxin C fragment does not remedy the deficiencies of the combination of references as a whole.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claim 45 under 35 U.S.C. 103(a).

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,

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